Reference: *Biol. Bull.*, **142**: 219-235. (April, 1972)

THE ROLE OF ULTRASTRUCTURE AND PHYSIOLOGICAL DIFFERENTIATION OF EPITHELIA IN AMINO ACID UPTAKE BY THE BLOODWORM, *GLYCERA*

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Soft-bodied marine invertebrates are able to remove amino acids and other small organic compounds from very dilute solution in the surrounding sea water. The evidence for the occurrence and potential significance of this phenomenon has been recently reviewed (Stephens, 1967, 1968, in press). It has been shown in a a number of cases that uptake of amino acids can occur directly across the body wall. This has been demonstrated by mechanically occluding the gut (Stephens, 1962, 1963, 1964; Chapman and Taylor, 1968) and can also be concluded from autoradiographic studies (Ferguson, 1967; Pequignat and Pujol, 1968; Little and Gupta, 1969).

In most invertebrates studied, as the concentration of amino acids is increased in the ambient medium, the rate of uptake also increases provided concentrations are low. At higher concentrations, the system mediating uptake becomes saturated and further increase in ambient concentration does not produce a corresponding increase in the rate of uptake. This system can be described adequately by Michaelis-Menten kinetics. The maximum velocity of uptake (V_{max}) and the ambient concentration at which the system is half-saturated (K_t) can be determined. The reported K_t values for most marine invertebrates ranges from 5×10^{-5} to 2×10^{-3} moles/liter and that of V_{max} from 10^{-7} to 10^{-5} moles/g/hour (Stephens, in press). Southward and Southward (1970) determined the K_t value for certain Pogonophora to be as low as 10^{-7} moles/liter.

The bloodworm, *Glycera dibranchiata,* has been shown to accumulate amino acids and creatine from the surrounding medium (Stephens, Van Pilsum and Taylor, 1965). Preston and Stephens (1969) and Preston (1970) have shown that coelomocytes of *Glycera* suspended in sea water concentrate amino acids very rapidly. Thus both the worm as an organism and at least some of its tissues have this capacity. Uptake by coelomocytes is correlated with the maintenance of large pools of free amino acids intracellularly and is presumably the mechanism whereby large differences between cells and extracellular fluid are achieved.

Glycera is a favorable animal for a further analysis of the pathway (or pathways) of entry of dissolved organic compounds. It is a large worm. The gut is suspended in an extensive, undivided coelom. Circulation of the hemoglobin-containing coelomocytes is accomplished by ciliary currents and there is no other circulatory system. Its comparatively simple organization and size facilitate experimental analysis. In particular, it is easy to obtain portions of the body wall

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free of other tissues for study. Klawe and Dickie (1957), and Clark (1962), give an account of the morphology and general biology of the worm. This paper is a report of the structure of the external and internal epithelia of the body wall and the way they contribute to the uptake of amino acids from the surrounding sea water and the coelomic fluid.

Materials and Methods

Animals were commercially collected in Maine and shipped by air to California. We purchased worms from local dealers as needed and maintained them in sea water at 10–15° C. In some cases, worms were maintained in water or in moist seaweed at lower temperatures.

For general histology and orientation, portions of worms were fixed in Bouin's solution and embedded in paraffin. Sections were stained with paraldehyde fuchsin and picro-indigo carmine (Gabe, 1953).

For electron microscopy, small pieces of body wall were fixed in 5% glutaraldehyde and postfixed in 2% osmium tetraoxide. Both fixatives were buffered to pH 7.2 by 0.1 molar phosphate buffer with or without the addition of 0.45 molar sucrose. Tissues were then dehydrated in an acetone series and embedded in Epon 812 (Luft, 1961).

Glass or diamond knives were used to cut sections on a Reichert OMU2 ultramicrotome. 1 to 2 μm sections stained with toluidine blue were studied under a light or phase microscope. Contrast of thin sections was enhanced by Karnovsky's (1961) and Reynolds' (1963) lead stain or by 2% phosphotungstic acid. Most satisfactory results were obtained with 2% aqueous uranyl acetate followed by Reynolds' lead citrate.

Electron micrographs were taken with a Zeiss EM 9A or 9S-2 microscope.

Worms prepared for scanning electron microscopy were quick frozen in isopentane chilled with liquid nitrogen and then freeze-dried by the moving gas method (Jensen, 1962). Dried tissue was coated with gold and carbon in a Varian vacuum evaporator, and examined in JEOL scanning electron microscope.

Morphology and amino acid composition of isolated cuticle was also studied. Large pieces of isolated cuticle were mounted on glass slides, air dried, and observed under a phase microscope. Worms were anesthetized by adding ethanol slowly to the sea water or using ether (Reed and Rudall, 1948). Cuticle was stripped from the body using fine forceps. The cuticle was then washed repeatedly in distilled water and kept overnight at 4° C in distilled water to remove adhering epithelial cells. Samples were checked by electron microscopy to ensure the absence of adhering cells. For determination of amino acid composition, the cuticle was hydrolysed in a sealed vial at 100° C in 6 N HCl. The HCl was evaporated off and the amino acid residues dissolved in a small amount of 80% ethanol. This solution was spotted on a cellulose thin-layer plate (20 × 20 cm MN-Polygram Cel #300, Brinkman Instruments, Inc.). The amino acids were separated two-dimensionally using the solvent systems described by Jones and Heathcote (1966). The plates were air dried and sprayed with 2% ninhydrin in absolute ethanol. Color spots were developed at room temperature in darkness (Clark, 1968).

C^{14}-labelled amino acids were used in the uptake studies. Concentrations of amino acid greater than 10^{-6} molar were obtained by adding C^{14} amino acids as

required. Whole worms were exposed to the labelled solutions in beakers. Isolated pieces of body wall were exposed as described below. All experiments were run at room temperature (21 ± 1° C). Both disappearance of radioactivity from the medium and appearance of radioactivity in the tissue were followed when possible. After a suitable time of exposure, whole worm or isolated pieces of body wall were washed in sea water and extracted with 80% ethanol or 5% cold trichloroacetic acid. Duplicate samples of sea water or tissue extract were mixed with 10 ml of a scintillation cocktail (2 volumes of a solution of 6 g PPO/liter of toluene and 1 volume of a detergent, Triton X-100) and counted in a Beckman CPM-100 scintillation system. Some of the samples from whole worm uptake experiments were dried on aluminum planchets and counted with a thin-window gas flow GM detector. All data are corrected as necessary for quenching and background.

Uptake of amino acids across the external and the internal surface of the body wall was examined using a Ussing chamber. The cross-section area of the windows through which the tissue was exposed to the solution in the two halves of the chamber was approximately 0.5 cm^2. Solution volume was 10 ml on each side. Pieces of body wall approximately 2 × 3 cm were isolated, rinsed in sea water and clamped between the halves of the chamber. C^{14}-labelled amino acid at a concentration of approximately 10^{-6} moles per liter (1 microcurie in 20 ml) was supplied on one side and sea water bathed the other. Radioactivity of an 80% ethanol extract of the tissue was determined after a suitable exposure time.

A simplified smaller chamber (Fig. 1A) consisting of a tube open at both ends with a broad base was used in further studies on the uptake kinetics of the external and the internal surface. The cross-section area of the chamber was approximately 0.6 cm^2. Volume of solutions used was reduced to 0.5 ml or less. Thus, radioactivity disappearing from the medium as well as that appearing in the tissue could be measured. A similar chamber (Fig. 1B) was modified by closing the top and providing entrance and exit ports for study of uptake for longer time periods.

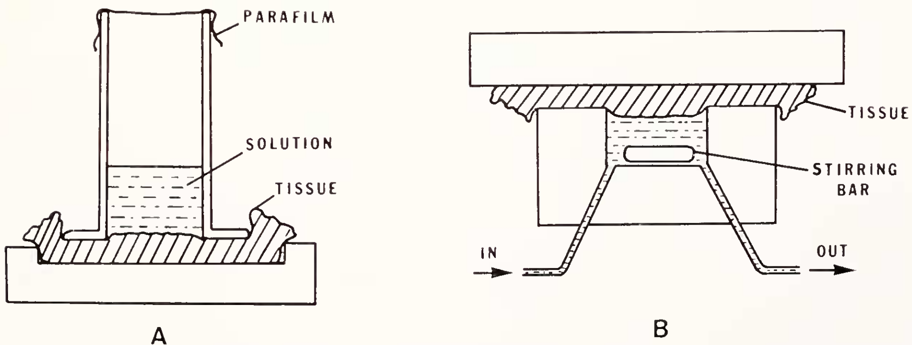
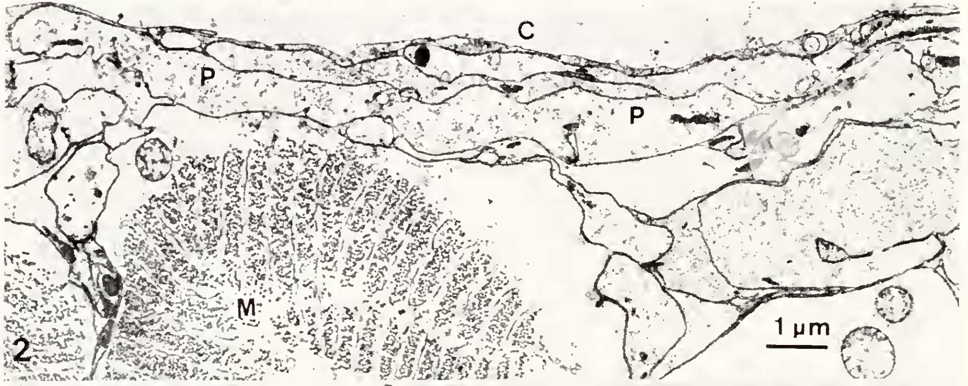
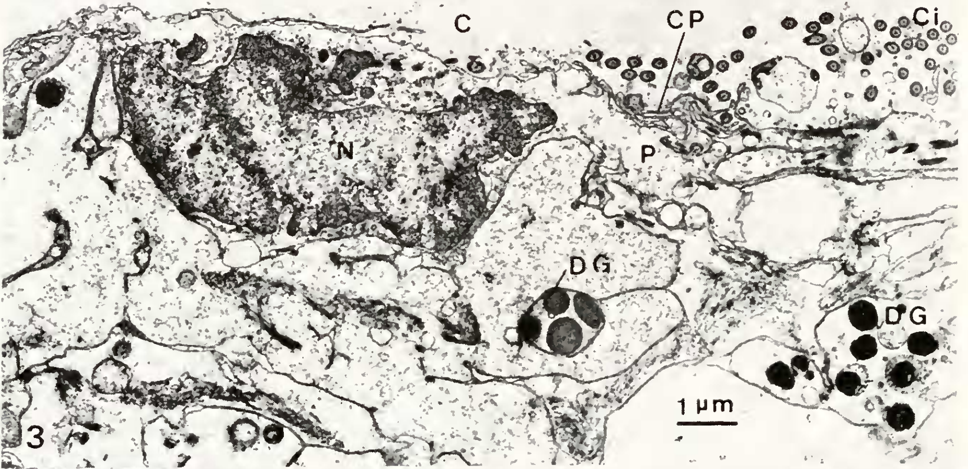
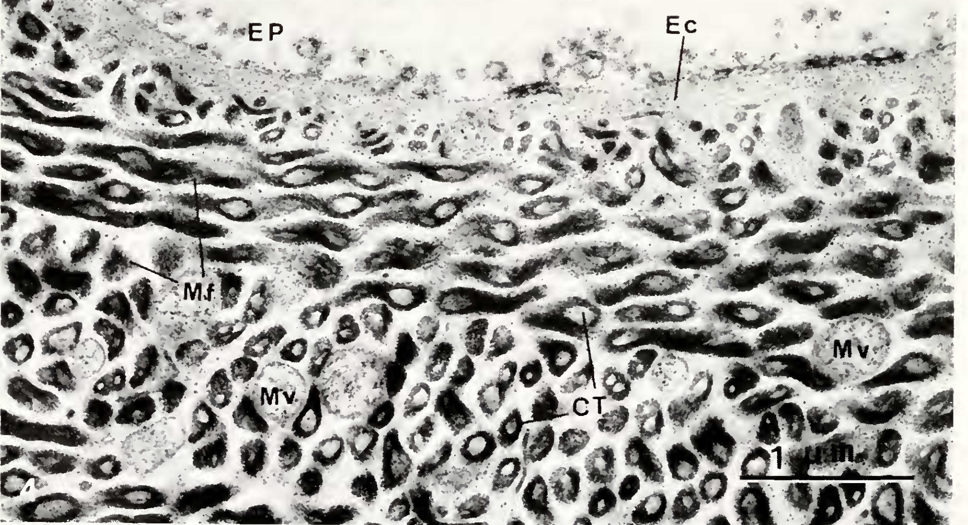


FIGURE 1. (A) Small chamber used in studies on the kinetics of amino acid uptake by the body wall of *Glycera*. Mixing of solution was achieved by mounting this preparation on a shaker. (B) Closed chamber used in studies on continuous uptake. Amino acid solution was passed through the chamber by a peristaltic pump and effluent solution was collected by a fraction collector. The small magnetic stirring bar was used for mixing in the chamber.







FIGURES 2–4.

Results

A. *Light microscopy*

The body wall of *Glycera* consists of two epithelial and two muscle layers. The outer, circular muscles form a complete ring around the inner, longitudinal muscles. The longitudinal muscles are arranged in four massive bundles due to the presence of the lateral parapodia, the dorsal septa and the ventral nerve cord. The peritoneum lines the coelomic surface of the longitudinal muscles. The epidermis attaches to the outside of the circular muscles by a basement membrane. It consists of columnar to pseudo-stratified columnar cells. A large number of goblet cells is scattered among the apical portion of the columnar cells. The contents of the goblet cells stain purple with paraldehyde-fuchsin which indicates the presence of mucopolysaccharides. The epidermis is covered by a non-cellular cuticle. Under the phase microscope, the cuticle has a laminated appearance. It stains blue with paraldehyde-fuchsin, suggesting the presence of collagen (Gabe, 1953).

Isolated cuticle, dried on glass slides, shows layers of fibers crossing at right angles. All fibers form a 45° angle with the long axis of the worm. Refractile tubules, interpreted as openings of the goblet cells, perforate the network of fibers at regular intervals.

B. *Electron microscopy*

The peritoneum which covers the coelomic surface of the longitudinal muscles, is composed of one to several layers of flattened cells (Fig. 2). In certain areas, ciliary bands and irregular cytoplasmic projections extend into the coelom (Fig. 3). In these cells, large membrane-bound electron-dense granules up to 1 μm in diameter are common. Mitochondria, Golgi bodies, endoplasmic reticulum and other organelles are rarely seen. In general, these epithelial cells have a very simple organization.

The structure of the epidermis and cuticle is much more complicated (Fig. 5). The cuticle ranges from 1 to 12 μm in thickness. It is thinnest over the parapodia and gills. The fibers observed and described at the light microscope level, appear as layers of highly ordered and densely packed tubules in electron micrographs (Figs. 4 and 6). Each layer is parallel to the surface but oriented perpendicularly to adjacent layers. Fifteen or more layers may occur (Figs. 5 and 6). Individual tubules measures 0.1 to 0.3 μm in diameter and are composed of about 5 nm thick fibers. The largest tubules occur in central layers where the lumen (or electron-transparent region) is clearly shown (Fig. 6). The tubules in the innermost and outer layers are smaller and look solid at times. Two per cent phosphotungstic acid provides optimum contrast and shows the tubular nature best (Fig. 4). A homogeneous epicuticular layer lies over the network of tubules, which in turn is

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FIGURE 2. A typical view of the coelomic lining of the muscular body wall of *Glycera dibranchiata;* C = coelom; M = muscle; P = peritoneum; Uranyl acetate and lead citrate stain.

FIGURE 3. Portion of coelomic lining showing irregular cytoplasmic extensions (CP) and cilia (Ci); C = coelom; DG = dense granule; N = nucleus; P = peritoneum; Uranyl acetate and lead citrate stain.

FIGURE 4. Apical portion of *Glycera* cuticle stained with 2% phosphotungstic acid to show the tubular nature of the collagen tubules (CT) and the microfibers (Mf); Ec = Epicuticle; EP = epicuticular particles; Mv = microvilli.

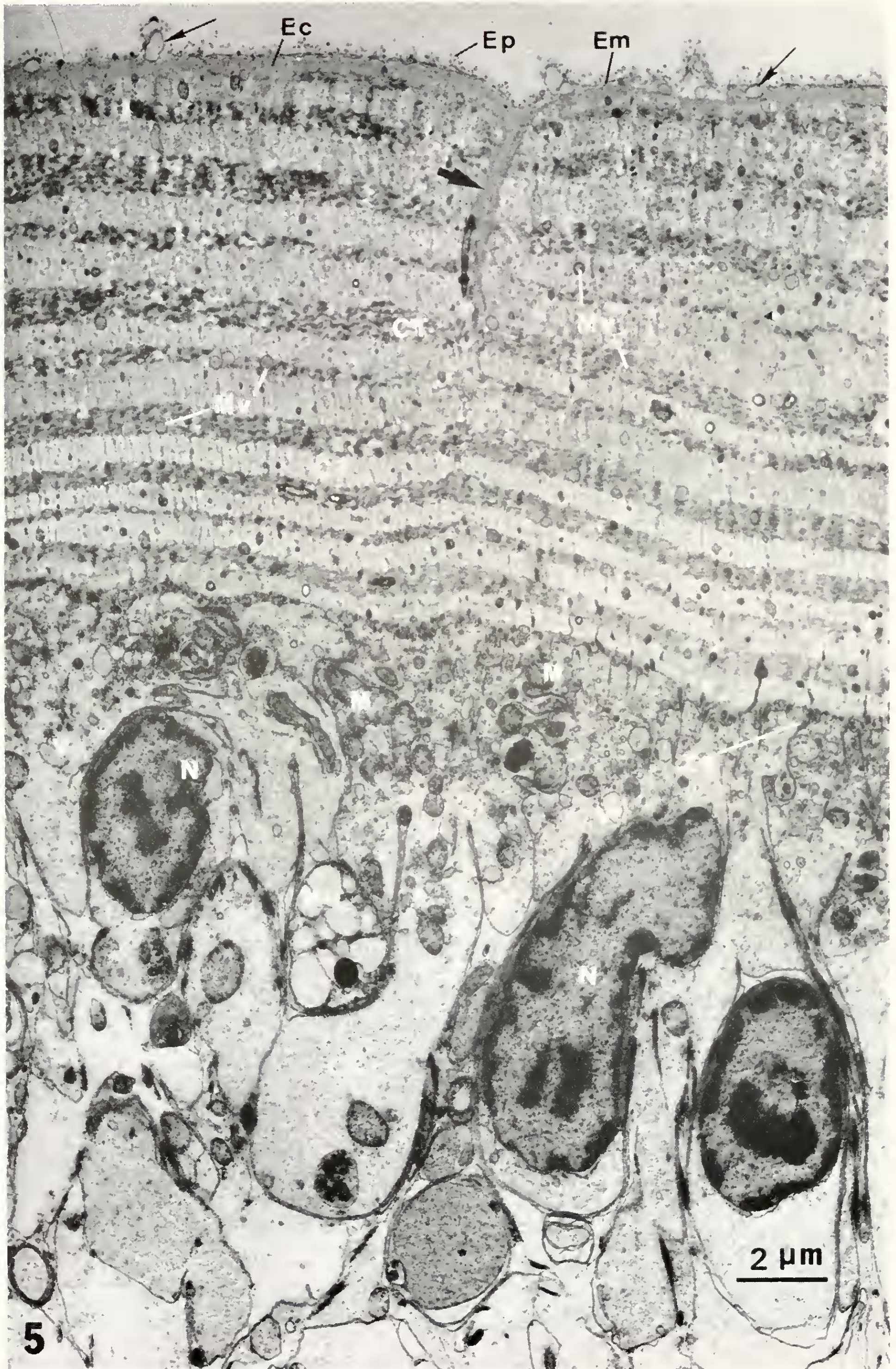

FIGURE 5.

outlined by an electron-dense epicuticular membrane (Fig. 5). Dense epicuticular particles about 0.1 to 0.2 μm in diameter stud the surface (Figs. 4, 5 and 6). The goblet cells are seen at various stages of secretion. The neck of each is surrounded by an invagination of the epicuticle (Fig. 5), which is seen as a refractile tubule in the light microscope. A large number of cytoplasmic extensions from the apical end of the columnar epithelial cells invades the cuticle proper (Fig. 7). Occasionally, they reach the surface but more typically terminate in the cuticle. These cytoplasmic extensions or microvilli measure up to 0.5 μm in diameter. Higher magnifications show the presence of particles about the size of free ribosomes in the microvilli, and bundles of tonofilaments at the more electron dense base. Mitochondria, endoplasmic reticulum, Golgi bodies, vesicles of various sizes and even nuclei occur at the apical portion of these columnar cells. In this region cells interdigitate with one another (Fig. 5). Zonula adherens from 0.2 to 0.5 μm long join the lateral sides of contiguous cells close to the cuticle. Electron dense striations occur in the cell junction below the zonula adherens which suggests the presence of septate desmosomes.

Scanning electron microscopy confirms the observations on the epicuticular particles, gland openings and the presence of some mucus material seen with the transmission electron microscope. Microorganisms have not been observed adhering to the general surface of *Glycera* by either method.

The increase of surface area due to the presence of microvilli was estimated by measuring the diameters or the width and lengths of the microvilli found in typical sections. The total circumference calculated was used as an index of their surface area. The width of epithelial cells was considered to be a measure of the surface area if the microvilli were absent. A comparison between the external and the internal surface was made. The relative measurements are expressed in arbitrary units in Table I. This estimation is only a rough one because of the complexity of the microvilli and the irregularity of the membrane foldings. As an average, the epidermal microvilli increase the surface area about thirteen times. The membrane foldings on the internal surface roughly double the surface area. Therefore, the epidermal surface has a total area about seven times that of the internal surface.

C. *Amino acid composition of the cuticle*

The general separation of amino acid residues of the cuticle hydrolysate was very good on the thin-layer chromatograms, but arginine did not separate completely from lysine and the glycine spot overlays hydroxyproline at high concentrations. A total of eighteen amino acids were identified from the chromatograms. Amino acids present in largest quantities were hyroxyproline, glutamic acid, lysine, glycine, alanine, and arginine. Proline, serine, leucine, threonine, valine, cystine and isoleucine were present in fairly high concentrations while

FIGURE 5. Cuticle and apical portions of *Glycera* epidermal cells. The numerous microvilli (Mv) penetrate the orthogonal layers of collagen tubules (CT) and occasionally come to the external surface (arrows). Note the concentration of organelles in the columnar cells (CC), the tangential section of a mucus cell (Mu) and part of its duct (arrow head). The duct is seen only as the invagination of the epicuticle (Ec) here: EM = epicuticular membrane; EP = epicuticular particles; M = mitochondron; ZA = zonular adherence; Karnovsky's stain.

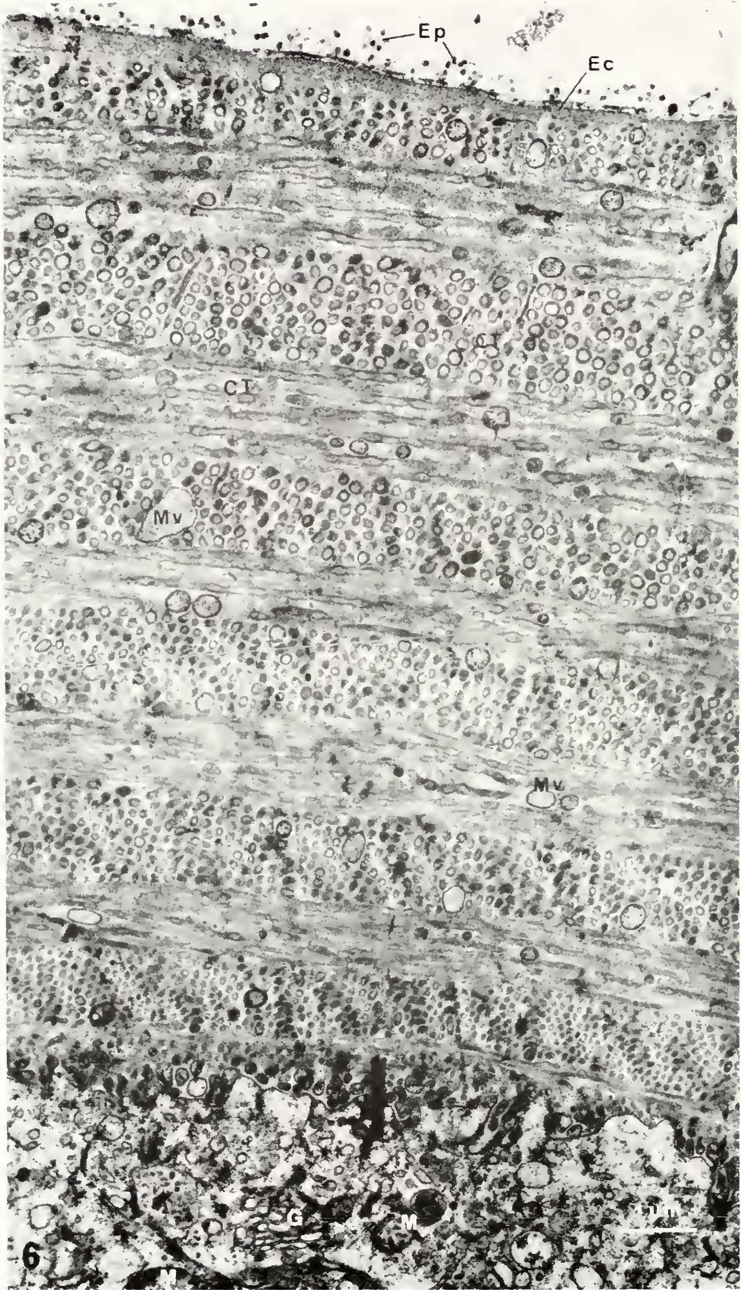

FIGURE 6. Orthogonal collagen tubules (CT) seen at longitudinal and cross sections. Note the diameter of the tubules are slightly smaller in those layers close to the epidermis and the epicuticle (Ec). Golgi apparatus (G), mitochondria (M), vesicles (V) and other organelles are found in the epidermal cells; Uranyl acetate and lead citrate stain.

TABLE I

Relative increase in surface area due to the presence of cytoplasmic extensions on the external and internal surface of Glycera *body wall (arbitrary units)*

Surface area of microvilli	Surface area of cells	Ratio	Average ratio
External surface			
2680	250	10.7	
1970	120	16.4	
1790	180	10.0	12.8
1680	100	16.8	
1020	100	10.2	
Internal surface			
120	150	0.8	
29	230	0.1	
0	200	0.0	0.82
22	200	0.1	
565	180	3.1	

phenylalanine, tyrosine, methionine, aspartic acid and histidine were present only in traces.

D. *Amino acid uptake*

Whole worms. When *Glycera* was placed in a solution of C^{14}-labelled amino acid, radioactivity decreased steadily in the medium and appeared in the worm. This observation was previously reported for uniformly labelled glycine and arginine (Stephens, Van Pilsum and Taylor, 1965). The present observations include six other amino acids. Rates of uptake were measured at concentrations ranging from 10^{-5} to 10^{-3} moles/liter of amino acid. The K_t values listed in Table II were obtained graphically using the Lineweaver-Burk linear transformation of the Michaelis-Menten equation.

After whole blood worms had been exposed for one hour to C^{14}-labelled nonmetabolizable amino acid analogs, 1-aminocyclopentane-1 carboxylic acid and α-aminoisobutyric acid, about 1% of the radioactivity which disappeared from the medium was recovered from the gut. Only 5% was located in the eversible

TABLE II

Kinetics of amino acid accumulation in whole worms, Glycera dibranchiata

	K_t – Molar	V_{max} – moles/hr/g worm
Alanine	2.08×10^{-3}	9.62×10^{-7}
Arginine	2.17×10^{-4}	1.61×10^{-7}
Aspartic acid	2.30×10^{-3}	1.56×10^{-7}
Glycine	2.52×10^{-4}	2.04×10^{-7}
Valine	1.20×10^{-3}	1.64×10^{-7}
Proline	3.68×10^{-4}	1.06×10^{-6}
Glutamic acid	3.93×10^{-4}	—
Phenylalanine	2.01×10^{-4}	—

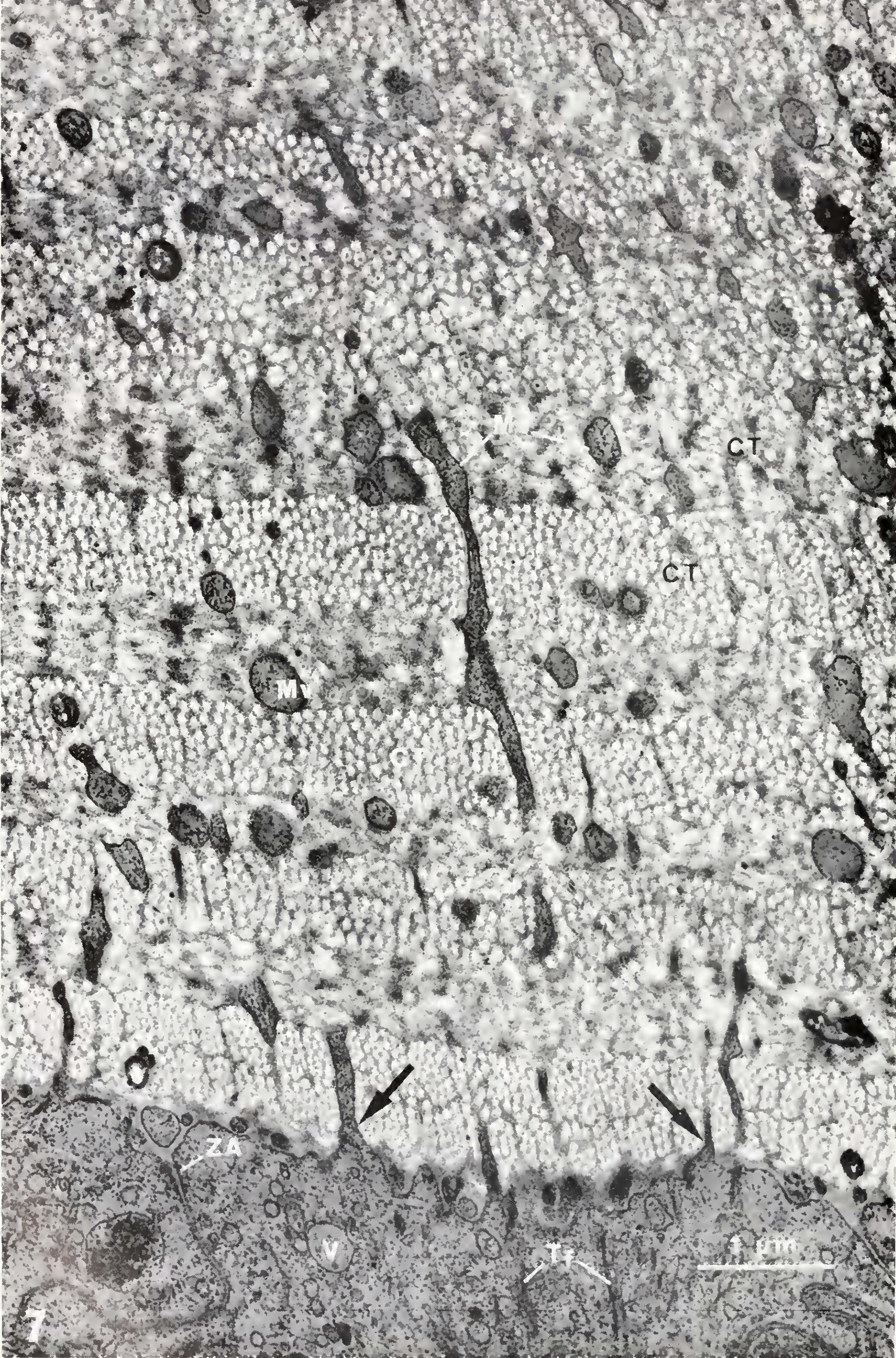

FIGURE 7.

proboscis and more than 75% was in the body wall. Thus, influx of amino acids occurs principally through the general body surface under these experimental conditions.

Isolated body wall. When isolated portions of the muscular body wall of *Glycera* are clamped into the Ussing chamber as indicated in Figure 1, C^{14}-labelled amino acids enter more rapidly across the external surface than the internal surface. Table III lists rates for glycine and phenylalanine, uptake across the external surface being markedly more rapid in both cases (13: 1 and 7: 1, respectively). After a fifteen minute exposure time, no detectable radioactivity was transferred from either chamber to the other. In fact, other observations continued for periods as long as 210 minutes showed no transfer of label.

Continuous uptake of amino acids was also followed. Pieces of body wall were clamped into a modified chamber such that only one surface of the tissue was exposed at a time. Amino acid solution was passed through the chamber at a

TABLE III

CPM in an alcohol extract of the body wall of Glycera *after exposure to 10^{-5} molar glycine-C^{14} or L-phenylalanine-C^{14} supplied to the internal or the external surface*

	External	Internal
Glycine	1580	125
	1350	65
	1000	105
Phenylalanine	530	82
	1040	141
	890	131

constant rate by using a peristaltic pump. A small magnetic stirrer was used for mixing in the chamber. The effluent solution was collected by a fraction collector and radioactivity was determined. Both surfaces of the isolated body wall were found to remove labelled amino acids at a constant rate over a period of three hours. Antibiotics were used in these prolonged observations to prevent bacterial contamination.

Kinetic parameters of the internal and external body wall surfaces were studied employing the simplified, small-volume chambers and using the nonmetabolizable amino acid, taurine. Taurine uptake by the external surface of isolated body wall shows kinetics similar to that of the whole worm (Fig. 8). K_t and V_{max} were evaluated using the Hofstee linear transformation (Dowd and Riggs 1965). The K_t has a value of $(6.3 \pm 1.7) \times 10^{-4}$ moles/liter. The V_{max} is $(8.53 \pm 1.44) \times 10^{-8}$ moles/hr/cm^2 (Fig. 9). The internal surface, however, behaves quite differently. The system takes up taurine but shows no sign of saturation at concentrations as high as 5×10^{-3} moles/liter. When the rate of uptake is plotted against medium concentration, a straight line passing through the origin is obtained (Fig. 8). A

FIGURE 7. Favorable section showing the microvilli (Mv) extending from the epidermal cells into the cuticle proper (arrows). Tonofilaments (Tf), zonular adherence (ZA) and various vesicles (V) can be seen; Karnovsky's stain.

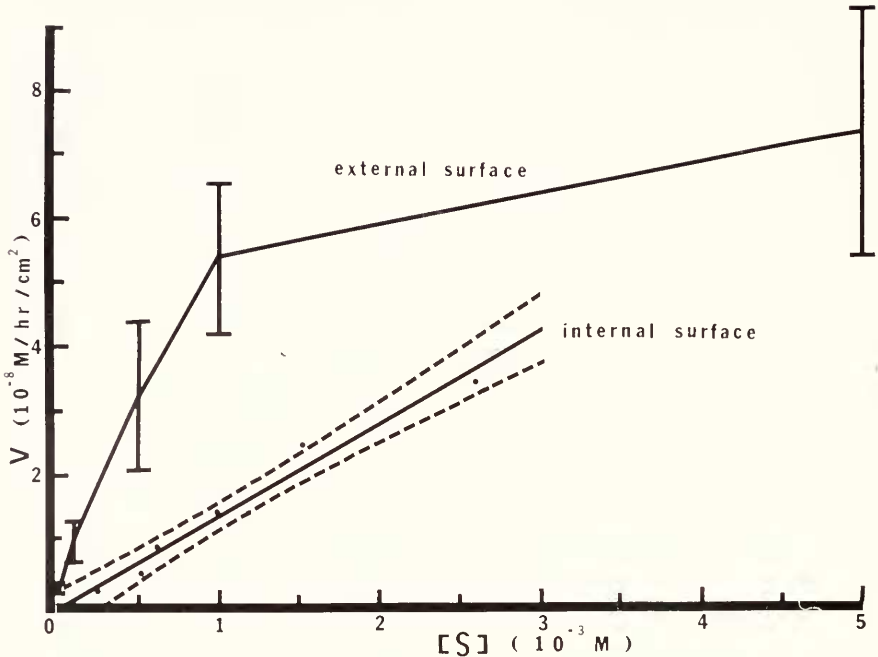

FIGURE 8. Velocity of taurine uptake by isolated pieces of *Glycera* body wall as a function of the medium concentration. The vertical bars indicate standard deviations. The broken lines show the 95% confidence interval of the linear regression calculated by the least squares method.

Hofstee plot results in a straight vertical line, which does not intercept the ordinate (Fig. 9). The rate of uptake is much lower than that shown by the external surface in agreement with the observations on glycine and phenylalanine reported above.

Discussion

The general organization of the body wall of *Glycera* is typical of that of errant polychaetes. The ultrastructure of the peritoneum is similar to that which lines the coelomic surface of the proventriculus of *Syllis* (Boilly, 1970). The cuticle of *Glycera* stains blue with paraldehyde-fuchsin which suggests the presence of collagen protein. Our qualitative amino acid analysis shows the presence of high concentrations of hydroxproline and glycine which is characteristic of both vertebrate and invertebrate collagen (Astbury, 1947; Watson and Smith, 1956; Watson, 1958). We believe the fine fibrils seen in the orthogonal tubules are actually collagen fibrils. The term "tubules" is used only as a descriptive term. This is the only example of collagen tubules we know. Our observations made on cuticle of *Nereis limnicoli* and *Stauronereis rudolphi* show solid fibers like those reported for *Syllis* (Boilly, 1970). The orthogonal arrangement of collagen fibers is common and has been described in cuticle of nemerteans, molluscs, echinoderms and the basement lamella of platyhelminthes, fish and amphibians as well as human

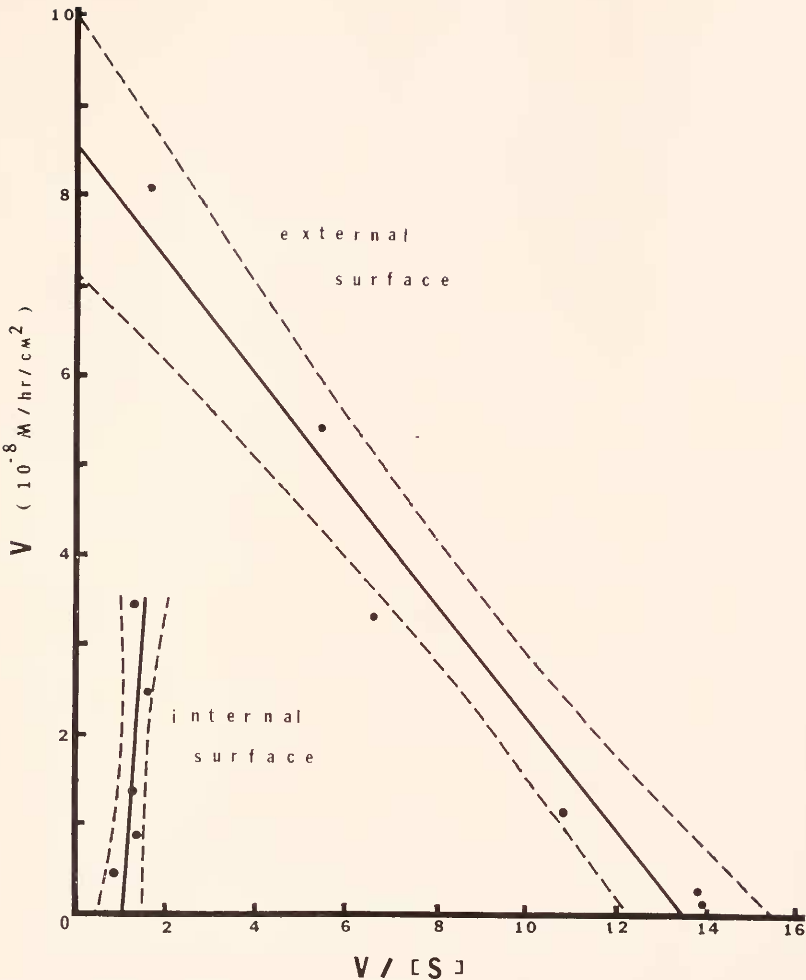

FIGURE 9. Hofstee's linear transformation of the Michaelis-Menten equation for taurine uptake across the external and internal surface of isolated pieces of body wall. The broken lines indicate the 95% confidence intervals. The lines were fitted by least squares method.

cornea (see references in Nadol, Gibbins and Porter, 1969; Lloyd, 1969; Menton and Eisen, 1970).

We found neither bacteria nor fungi associated with the external surface of *Glycera* in any scanning or transmission electron micrographs. However, small dense epicuticular particles were routinely present. Similar particles have been observed in all oligochaetes studied (Potswald, 1971). Several hypotheses concerning their nature and origin have been advanced (Reed and Rudall, 1948; Coggeshall, 1966). It seems clear now that they are derived from the tips of the epidermal microvilli (Hess and Menzel, 1967; Potswald, 1971). However, the

function of these particles is virtually unknown. We merely report their presence in *Glycera.*

The presence of epidermal microvilli is very common in soft bodied invertebrates. Recent reports of microvilli in other groups include the platyhelminthes (MacRae, 1967), the molluscs (Lloyd, 1969), the pogonophorans (Little and Gupta, 1969) and the echinoderms (Menton and Eisen, 1970). Their presence is often accompanied by a cuticle or mucilagenous material or both. Little is known about the function of these microvilli. Many speculations have been made. Since they are in direct contact with the cuticle or mucus, a role in secretion and maintenance of these extracellular materials has been suggested. (Lane, 1963; Coggeshall, 1966; Potswald, 1971). Potswald (1971) observed the temporary elaboration of numerous microvilli during regeneration of the cuticle of an oligochaete. We wish to focus attention on the possible role of microvilli in absorption of material from the environment. Uptake of small organic compounds by free-living marine invertebrates has been well documented (Stephens, 1968). The resemblance in ultrastructure between the epidermal microvilli of invertebrates and the vertebrate intestinal brush border has been pointed out by many authors (Lane, 1963; Nørrevang, 1965; Little and Gupta, 1969; Potswald, 1971).

We have demonstrated that whole *Glycera* worms accumulate neutral, acidic and basic amino acids. The K_t's for eight different amino acids vary from 2×10^{-4} to 2×10^{-3} moles/liter. These values fall in the upper range of reported K_t values (5×10^{-5} to 2×10^{-3}) for most marine invertebrates. Stephens (in press) and Southward and Southward (1970) review the evidence and conclude that K_t values for marine organisms are related to the free amino acid concentrations in their normal habitats. This generalization applies to *Glycera* which live in muddy sediments (Klawe and Dickie, 1957) where dissolved organic substances are likely to be abundant.

Passage of amino acids into the body wall occurs more rapidly across the epidermis than the peritoneum. The microvilli on the external surface of *Glycera* provide a surface area about seven times greater than that of the internal surface (13.8: 1.8). This ratio roughly agrees with the ratio of influx rates determined by the Ussing chamber experiments. Probably the agreement is accidental since examination of Figure 8 shows that the ratio would depend on the concentration of amino acids present in the medium.

Further characterization of these two surfaces by the kinetic studies shows that the influx across the internal surface of *Glycera* body wall is directly proportional to the medium concentration, over a wide range, suggesting entry by passive diffusion. The apparent passive behavior of the peritoneum regarding amino acid influx is interesting in connection with the distribution and maintenance of intracellular amino acid pools. Preston and Stephens (1969) and Preston (1970) have shown that the high amino acid concentrations in *Glycera* coelomocytes are due to the ability of these circulating cells to accumulate such compounds. The interaction of passive and facilitated distribution of amino acids between the coelomic cells, coelomic fluid and the body wall remains to be described in detail.

In contrast to the internal surface, the external surface shows saturable kinetics which characterize other mediated transport systems. The K_t for taurine agrees well with the K_t values for other amino acids determined for the whole worms

(Table II). Therefore, the ultrastructural and uptake studies lead to the conclusion that differentiation to increase the surface area and the intrinsic properties of the cell membrane are both important in the transport of dissolved organic molecules across the epidermis of *Glycera*.

We would like to express our thanks to Dr. A. Loeblich II for the use of the JEOL microscope; Mr. R. MacAdam and Miss J. Kiethe for technical assistance in scanning electron microscopy; Mr. J. Biela for help in construction of an experimental chamber.

This work was supported in part by the U. S. Public Health Service grant No. GM 12889 and the Graduate School of the University of California.

Summary

1. The epidermis of *Glycera* consists of interdigitating columnar cells and mucus secreting goblet cells. Both types of cells are covered by a collagenous cuticle, 1 to 12 μm thick.

2. The cuticle is made up of layers of orthogonally arranged tubules in an amorphous matrix. Individual tubules measures 0.1 to 0.3 μm in diameter and are composed of collagen fibers about 5 nm thick.

3. Microvilli extending from the columnar epithelial cells into the cuticle increase the surface area of these cells about 13 times.

4. The peritoneum which covers the coelomic surface of the body wall is composed of one to several layers of flattened cells. Irregular cytoplasmic projections double the surface area.

5. *Glycera* whole animals take up eight different amino acids by saturable systems. The K_t values range from 2×10^{-4} to 2×10^{-3} moles/liter, which fall in the upper range of reported K_t values for marine invertebrates.

6. Influx of amino acids occurs across both surfaces of isolated pieces of body wall. The kinetic characteristics of transport across the epidermis are similar to those of the whole worms.

7. Both the ultrastructure and the physiological differentiation of the epithelial cells are shown to be important in the transport of amino acids across *Glycera* body wall.

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